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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No. 0052552

MEMORANDUM

DATE: June 10, 2004

SUBJECT: **SPIRODICLOFEN:** Report of the Cancer Assessment Review Committee
PC Code: 124871

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Jessica Kidwell

TO: Yung Yang, Toxicologist
Toxicology Branch, Health Effects Division (7509C)

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Rita Kumar, PM
Insecticide/Rodenticide Branch, Registration Division (7505C)

The Cancer Assessment Review Committee met on May 5, 2004 to evaluate the carcinogenic potential of Spirodiclofen. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

RC
07/04

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

SPIRODICLOFEN

PC Code: 124871

FINAL
June 10, 2004

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Yung G. Yang, Ph.D.

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

<u>COMMITTEE MEMBERS IN ATTENDANCE:</u> (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke

William Burnam

Marion Copley

Kit Farwell

Abdallah Khasawinah

Nancy McCarroll

Esther Rinde

Linda Taylor

<u>NON-COMMITTEE MEMBERS IN ATTENDANCE</u> (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John Pletcher, Consulting Pathologist — See attached sheet

Lori Brunsman, Statistical Analysis

<u>OTHER ATTENDEES:</u> Rita Kumar (RD/IRB), Yong-Hwa Kim (visiting scholar, HED/RRB1), Susan Makris (HED/TB), PV Shah (HED/RAB1), Karen Whitby (HED/RAB1)
Conference call with Canada's PMRA: Brenda Linke, Benny Ling, Katie Calp, and Scott Hancock

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

On May 5, 2004, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Spirodiclofen.

Yung Yang of Toxicology Branch presented the chronic toxicity/carcinogenicity studies in Wistar rats and CD1 mice by describing the experimental design, reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, adequacy of the dose levels tested, and presenting the weight of the evidence for the carcinogenicity of spirodiclofen. Dr. Yang also discussed the toxicology, metabolism, mutagenicity, structure-activity relationships as well as mode of action findings.

Spirodiclofen was administered in the diet to groups of Wistar rats (50/sex/dose) at dose levels of 0, 50, 100, 350 or 2500 ppm (0, 2.0, 4.1, 14.7 or 110.1 mg/kg/day for males; 0, 2.9, 5.9, 19.9 or 152.9 mg/kg/day for females) for 107 weeks. An additional 10 rats/sex/dose were designated for interim sacrifice at week 54. Spirodiclofen was administered in the diet to CD-1 mice (50/sex/dose) at dose levels of 0, 25, 3500 or 7000 ppm (0, 4.1, 610 or 1216 mg/kg/day for males; 0, 5.1, 722 or 1495 mg/kg/day for females) for 18 months.

The CARC concluded that Spirodiclofen showed evidence of carcinogenicity based on the following:

- Male rats had a significant increasing trend at $p<0.01$, and a significant difference in the pair-wise comparison of the 2500 ppm dose group with the controls at $p< 0.05$, for testicular Leydig cell adenomas. The incidence of Leydig cell adenomas was 2/34 (6%), 1/35 (3%), 0/43 (0%), 4/35 (11%), and 10/43 (23%) for the control, 50, 100, 350, and 2500 ppm dose groups, respectively. The incidence of Leydig cell adenomas of 23% for the high dose group is outside the historical control range of the performing laboratory (2-8%). The incidence of Leydig cell adenomas for the 350 ppm dose group (4/35 (11%) (censored); 4/50 (8%), uncensored), although not statistically significant, was just within the boundary of the historical control range (2-8%) and was considered to be biologically significant. This is supported by an increase (not statistically significant) in focal Leydig cell hyperplasia at 350 ppm **Therefore, the CARC considered the increase in Leydig cell adenomas at 350 and 2500 ppm to be treatment-related.**

- Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 2500 ppm dose group with the controls, for uterine adenocarcinomas and combined adenomas and/or adenocarcinomas, all at $p<0.01$. The incidence of uterine adenocarcinomas was 4/47 (9%), 5/48 (10%), 3/46 (7%), 2/46 (4%), 14/47 (30%) for the control, 50, 100, 350, and 2500 ppm dose groups respectively. The incidence of combined adenomas and adenocarcinomas was 4/47 (9%), 5/48 (10%), 3/46 (7%), 3/46

(7%), 14/47 (30%) for the control, 50, 100, 350, and 2500 ppm dose groups respectively. The incidence of adenocarcinomas at the high dose (30%) was outside the laboratory historical control range of 2-10% for adenocarcinomas. **The CARC considered the uterine tumors (adenocarcinomas) at the high dose to be treatment-related.**

- **The CARC considered dosing at the high dose (2500 ppm) to be adequate and not excessive based on body weight decrease, changes in clinical chemistry, and histopathological findings.** Significant decreases in body weights were observed in the 2500 ppm group of both sexes compared to controls (↓8-10% for males up to weeks 101; ↓6-7% for females up to weeks 53). Body weight gains were decreased at 2500 ppm of both sexes up to week 3 and recovered thereafter. Clinical chemistry findings in both sexes at 2500 ppm included increased alkaline phosphatase, and decreases of cholesterol and triglyceride levels. Significantly increased thyroxine (T4) levels were observed in 2500 ppm males at weeks 53 and 105. Increased thyroid stimulating hormone (TSH) were observed at 2500 ppm of both sexes, but the statistical significance was observed only in females at weeks 79 and 105. Histopathological findings included vacuolated enterocytes in the jejunum (both sexes), vacuolation in Zona fasciculata cells of the adrenal cortex (males only), increased portion of ovarian stroma, and increased incidence of uterus nodules (females) at 2500 ppm.

- Male mice had significant increasing trends in liver adenomas and adenomas and/or carcinomas combined, both at $p<0.01$. There was a significant increasing trend in liver carcinomas at $p<0.05$. There were significant differences in the pair-wise comparisons of the 3500 and 7000 ppm dose groups with the controls for liver adenomas, both at $p< 0.05$. There were significant differences in the 3500 ppm dose group at $p<0.05$ and in the 7000 ppm dose group at $p<0.01$ with the controls for liver adenomas and/or carcinomas combined. The incidence of combined adenomas and carcinomas was 1/46 (2%), 1/50 (2%), 8/47 (17%), and 10/48 (21%) for the control, 25, 3500, and 7000 ppm dose groups, respectively. The incidences of combined liver adenomas and carcinomas of 17% and 21% for the 3500 ppm and 7000 ppm dose groups, respectively, are outside the historical control range of the performing laboratory (4-14%). **The CARC considered the increase in adenomas and combined adenomas and/or carcinomas in the liver at the top two doses to be treatment-related.**

- Female mice had a significant increasing trend, and a significant difference in the pair-wise comparison of the 3500 ppm dose group with the controls, for liver adenomas and/or carcinomas combined, both at $p<0.05$. The incidence of combined adenomas and/or carcinomas was 0/49 (0%), 0/50 (0%), 5/48 (10%), and 3/47 (6%) for the control, 25, 3500, and 7000 ppm dose groups, respectively. The incidences of combined liver adenomas and carcinomas of 10% (statistically significant) and 6% (not statistically significant) for the 3500 ppm and 7000 ppm dose groups, respectively, are outside the historical control range of the performing laboratory (0-2%). **The CARC considered the increase in combined adenomas and/or carcinomas in the liver at the top two doses to be treatment-related.**

- The CARC considered the high dose of 7000 ppm, a limit dose, in the mouse carcinogenicity to be adequate and not excessive. This was based on observations of increased organ weights (liver, adrenal gland, and testes) and histopathological findings (vacuolation of adrenal cortex, hepatocytomegaly, and testicular hypertrophy/hyperplasia of interstitial cells).

- There is no mutagenicity concern for spirodiclofen.

- There are no SAR data for spirodiclofen.

- The data, as presented, do not support a mode of action for spirodiclofen.

In accordance with the EPA Proposed Guidelines for Carcinogen Risk Assessment (July 1999), the Committee classified Spirodiclofen as **"Likely to be Carcinogenic to Humans"**, based on tumors seen in both sexes of two species (male rat testicular Leydig cell tumors (high dose), female rat uterine tumors (high dose), male and female mouse liver tumors (multiple doses)).

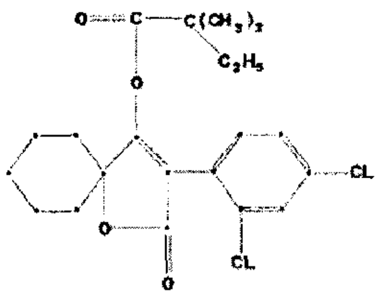
The CARC recommended that a low dose extrapolation model be applied to the experimental animal tumor data and that quantifications of risk be estimated for male rat testes, female rat uterine, and male and female mouse liver tumors for Spirodiclofen. The most potent unit risk will be used for the purpose of lifetime cancer risk assessment by the Agency.

I. Introduction

On May 5, 2004, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of spirodiclofen. This was the first time that this compound was assessed for carcinogenicity by the CARC.

II. Background Information

Chemical Name:	Spirodiclofen (BAJ 2740)
Empirical Formula:	$C_{21}H_{24}C_{12}O_4$
Molecular Weight:	411.3
CAS Registry No.:	148477-71-8
PC Code:	124871
Structure:	



Spirodiclofen (3-(2,4-Dichlorophenyl)-2-oxo-1-oxaspiro,<4.5> dec-3-en-4-yl ester 2,2-dimethylbutanoic acid) is a foliar acaricide belonging to a new chemical class of Tetronic acids. BAJ 2740 is the research identification number for the spirodiclofen technical. Its mode of action is described as a lipid biosynthesis inhibitor which interferes with biochemical processes associated with mite development.

The formulation (Envidor 2SC) is a suspension concentrate which contain 2.0 lb active ingredient per gallon of the miticide spirodiclofen. The proposed crop use sites are citrus, pome fruit, stone fruit and tree nuts. The highest rate of application is to the tree nuts at 0.53 lb a.i./A. It may only by applied one time per season. Applications will occur via airblast machinery. It may not be applied via any type of irrigation system. It may not be applied aerially. It may not be used in any enclosed structures such as greenhouses or planthouses. For pesticide handlers, there will be dermal and inhalation exposure. There is a 12 hour Restricted Entry Interval (REI) and a 7 day preharvest interval for all the proposed crops. There will be dermal postapplication exposure to agricultural workers and the post-application inhalation exposure will be negligible. There are no proposed residential uses evident on the proposed label.

III. Evaluation of Carcinogenicity Studies

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

References: Wirnitzer, U., Bach, U. and Hartmann, E. (2000). BAJ 2740: Combined Study on Chronic Toxicity and Carcinogenicity in Wistar Rats (Dietary Administration over 2 Years). Bayer AG Department of Toxicology, Friedrich-Ebert-Strasse 217-333 D-42069 Wuppertal, Germany. Bayer AG Report No. 30399, Bayer AG Study No. T7061640. Bayer AC Report No. 110516. Study completion date, October 27, 2000. MRID 45696808. Unpublished.

Wirnitzer, U. and Hartmann, E. (2002) Supplemental Submission to AC Report No. 110516: BAJ 2740 Combined Study on Chronic Toxicity and Carcinogenicity in Wistar Rats (Dietary Administration Over 2 Years). Bayer AG Department of Toxicology, Germany. Study Completion date, March 7, 2002. MRID 45696809. Unpublished.

A. Experimental Design

Groups of Wistar rats (50/sex/dose) were fed spirodiclofen at dose levels of 0, 50, 100, 350 or 2500 ppm (0, 2.0, 4.1, 14.7 or 110.1 mg/kg/day for males; 0, 2.9, 5.9, 19.9 or 152.9 mg/kg/day for females) for 107 weeks. An additional 10 rats per sex per dose were designated for interim sacrifice at week 54.

B. Discussion of Mortality and Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated a statistically significant decreasing trend with increasing doses of Spirodiclofen in male rats (Table 1). There was a statistically significant increasing trend in mortality with increasing doses of Spirodiclofen in female rats (Table 2) (Memo, L. Brunsman, 4/7/2004, TXR No. 0052471). The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Table 1. Spirodiclofen - Wistar Rat Study
Male Mortality Rates+
Cox or Generalized K/W Test Results

Dose (ppm)	Weeks					
	1-26	27-53	54[i]	54-78	79-107[f]	Total
0	1/60	4/59	10/55	4/45	9/41	18/50 (36)*n
50	1/60	1/59	10/58	3/48	15/45	20/50 (40)
100	2/60	0/58	10/58	1/48	10/47	13/50 (26)
350	0/60	1/60	10/59	2/49	16/47	19/50 (38)
2500	1/60	1/59	10/58	2/48	5/46	9/50 (18)*n

+Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 54.
[f]Final sacrifice at week 107.
( )Percent.
n: Negative trend or negative change from control.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Spirodiclofen - Wistar Rat Study
Female Mortality Rates+
Cox or Generalized K/W Test Results

Dose (ppm)	Weeks					
	1-26	27-53	54[i]	54-78	79-109[f]	Total
0	1/60	1/59	10/58	1/48	16/45a	19/48 (40)*
50	0/60	0/60	10/60	2/50	16/48	18/50 (36)
100	0/60	1/60	10/59	3/49	14/45b	18/49 (37)
350	0/60	1/60	10/59	3/49	11/46	15/50 (30)
2500	0/60	0/60	10/60	5/50	19/45	24/50 (48)

+Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 54.
[f]Final sacrifice at week 107.
( )Percent.
a: Two accidental deaths at week 83, dose 0 ppm.
b: One accidental deaths at week 79, dose 100 ppm.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analysis

As shown in Table 3, male rats had a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 2500 ppm dose group with the controls at $p < 0.05$, for testes leydig cell adenomas (2/1/0/4/10*). Concurrently, increased incidence of Leydig cell hyperplasia (4/4/4/7/19**) was observed in high dose rats.

Female rats, as shown in Table 4, had significant increasing trends, and significant differences in the pair-wise comparisons of the 2500 ppm dose group with the controls, for uterine adenocarcinomas and adenomas and/or adenocarcinomas combined, all at $p < 0.01$. The majority of the adenocarcinoma (11 out of 14) was found in females which died or had to be sacrificed before the termination of the study. The pathology report also indicated that many of the adenocarcinomas had metastasized by invasion and intra-abdominal spread into various organs of the abdominal cavity such as ovaries, liver, spleen, pancreas, mesenteric lymph node, kidney, lung, and bone marrow.

Historical Control Data

Historical control data from six in-house 24-25 months studies conducted in 1994-1996 (MRID 45696809) showed that rates for Leydig cell adenoma in testis are 2-8%, rates for Leydig cell hyperplasia are 2-10%, and rates for uterus adenocarcinoma are 2-10%.

Table 3. Spirodiclofen - Wistar Rat Study

Male Testes Tumor Rates+

Peto's Prevalence Test Results

	Dose (ppm)				
	0	50	100	350	2500
Adenomas (%) p =	2/34 (6) 0.0001**	1/35 (3) -	0/43 (0) -	4/35 (11) 0.1971	10[a]/43 (23) 0.0185*

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor. Excludes interim sacrifice animals.

[a]First adenoma observed at week 98, dose 2500 ppm.

Interim sacrifice animals are not included in these analyses. There were no tumors in any interim sacrifice animals.

Note: The statistical analyses of the male rats were based upon Peto's prevalence test.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Spirodiclofen - Wistar Rat Study
Female Uterine Tumor Rates+
Peto's Prevalence Test Results

	Dose (ppm)				
	0	50	100	350	2500
Adenomas (%)	0/29 (0)	0/32 (0)	0/31 (0)	1[a]/35 (3)	0/26 (0)
p =	-	-	-	0.1813	-
Adeno-carcinomas (%)	4/47 (9)	5/48 (10)	3/46 (7)	2/46 (4)	14[b]/47 (30)
p =	0.0001**	0.2915	-	-	0.0058**
Combined (%)	4/47 (9)	5/48 (10)	3/46 (7)	3/46 (7)	14/47 (30)
p =	0.0002**	0.2915	-	-	0.0058**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor. Excludes interim sacrifice animals.
[a]First adenoma observed at week 109, dose 350 ppm.
[b]First adenocarcinoma observed at week 76, dose 2500 ppm.
Note: The statistical analyses of the female rats were based upon Peto's prevalence test.
Interim sacrifice animals are not included in these analyses. There were no tumors in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Findings in Reproductive System

In males, increased incidence of focal Leydig cell hyperplasia was observed in high dose male rats (4/4/4/7/19**). No significant findings were observed in the epididymides, prostate, and seminal vesicle glands.

In females, increased incidence of uterus nodules were observed in terminal necropsy; however, no significant non-neoplastic lesions were observed in uterus.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The CARC considered dosing at the high dose (2500 ppm) to be adequate and not excessive based on body weight decrease, clinical chemistry, and histopathological findings. Significant decreases in body weights were observed in the 2500 ppm group of both sexes compared to controls (↓8-10% for males up to weeks 101; ↓6-7% for females up to weeks 53). Body weight gains were decreased at 2500 ppm of both sexes up to week 3 and recovered thereafter. Significant increases of alkaline phosphatase (APh) and decreases of cholesterol and triglyceride levels (not statistically significant) were observed in both sexes at 2500 ppm at all test points. Significantly increased thyroxine (T4) levels were observed in 2500 ppm males at weeks 53 and 105. Increased thyroid stimulating hormone (TSH) were observed at 2500 ppm of both sexes but the statistical significance was observed only in females at weeks 79 and 105. Gross and histopathology examinations showed an increased incidence of vacuolated enterocytes in the jejunum (both sexes), increased incidence and severity of vacuolation in Zona fasciculata cells of the adrenal cortex (males only), increased portion of ovarian stroma, and increased incidence of uterus nodules (females) at 2500 ppm. Increased incidence of treatment related neoplastic findings in reproductive organs of males (testes) and females (uterus) were observed at the 2500 ppm group.

2. Carcinogenicity Study in Mice

Reference: Wahle, B.S. (2000) Technical Grade BAJ 2740: An Oncogenicity Testing Study in the Mouse. Bayer Corporation Agriculture Division, Toxicology, Stilwell, KS. Laboratory Project Study ID No. 97-271-LV. Bayer Corporation Report No. 109626. July 21, 2000. MRID 45696724. Unpublished.

A. Experimental Design

CD-1 mice (50/sex/dose) were fed spirodiclofen at dose levels of 0, 25, 3500 or 7000 ppm (0, 4.1, 610 or 1216 mg/kg/day for males; 0, 5.1, 722 or 1495 mg/kg/day for females) for 18 months.

B. Discussion of Tumor Data

Survival Analysis

There were no statistically significant incremental changes in mortality with increasing doses of Spirodiclofen in male or female mice. See Table 5 for male mouse mortality test results and Table 6 for female mouse mortality test results. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Table 5. Spirodiclofen - CD-1 Mouse Study
Male Mortality Rates⁺
Cox or Generalized K/W Test Results

Dose (ppm)	Weeks			
	1-26	27-52	53-81	Total
0	0/50	4/50	8/46	12/50 (24)
25	0/50	0/50	14/50	14/50 (28)
3500	1/50	2/49	7/47	10/50 (20)
7000	1/50	1/49	12/48	14/50 (28)

+Number of animals that died during interval/Number of animals alive at the beginning of the interval.
fFinal sacrifice at week 80.
( )Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Spirodiclofen - CD-1 Mouse Study
Female Mortality Rates+
Cox or Generalized K/W Test Results

Dose (ppm)	Weeks			
	1-26	27-52	53-81	Total
0	1/50	0/49	11/49	12/50 (24)
25	0/50	0/50	14/50	14/50 (28)
3500	2/50	0/48	10/48	12/50 (24)
7000	1/50	2/49	11/47	14/50 (28)

+Number of animals that died during interval/Number of animals alive at the beginning of the interval.
fFinal sacrifice at week 80.
( )Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analysis

Male mice had significant increasing trends in liver adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$(Table 7). There was a significant increasing trend in liver carcinomas at $p < 0.05$. There were significant differences in the pair-wise comparisons of the 3500 and 7000 ppm dose groups with the controls for liver adenomas, both at $p < 0.05$. There were significant differences in the 3500 ppm dose group at $p < 0.05$ and in the 7000 ppm dose group at $p < 0.01$ with the controls for liver adenomas and/or carcinomas combined.

Female mice had a significant increasing trend, and a significant difference in the pair-wise comparison of the 3500 ppm dose groups with the controls, for liver adenomas and/or carcinomas combined, both at $p < 0.05$ (Table 8).

The statistical analyses of the male and female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons.

Historical Control Data

The historical control data from literature review for combined hepatocellular adenoma and carcinoma historical suggest a rate of 0-9.6% in males (n=499) and 0-2.7% in females (n=497) in nominal 18-month studies. Data from five in-house control studies conducted 1989-1998 showed a rate for the combined hepatocellular neoplasms of 4-14% in males (n=250) and 0-2% in females (n=250).

Table 7. Spirodiclofen - CD-1 Mouse Study
Male Liver Tumor Rates+
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	25	3500	7000
Adenomas (%) p =	0/46 (0) 0.0013**	0/50 (0) 1.0000	5/47 (11) 0.0295*	6[a]/48 (12) 0.0151*
Carcinomas (%) p =	1/46 (2) 0.0244*	1/50 (2) 0.7314	3/47 (6) 0.3166	5[b]/48 (10) 0.1118
Combined (%) p =	1/46 (2) 0.0002**	1/50 (2) 0.7314	8/47 (17) 0.0165*	10[c]/48 (21) 0.0047**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 72, dose 7000 ppm.
[b]First carcinoma observed at week 77, dose 7000 ppm.
[c]One animal in the 7000 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. Spirodiclofen - CD-1 Mouse Study
Female Liver Tumor Rates+
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	25	3500	7000
Adenomas (%)	0/49 (0)	0/50 (0)	3/48 (6)	1[a]/47 (2)
p =	0.1701	1.0000	0.1173	0.4896
Carcinomas (%)	0/49 (0)	0/50 (0)	2[b]/48 (4)	2/47 (4)
p =	0.0661	1.0000	0.2423	0.2371
Combined (%)	0/49 (0)	0/50 (0)	5/48 (10)	3/47 (6)
p =	0.0244*	1.0000	0.0266*	0.1135

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 77, dose 7000 ppm.
[b]First carcinoma observed at week 80, dose 3500 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Liver Lesions

In the liver, increased incidence and severity of hepatocytomegaly was observed in males (2/50, 6/50, 17/50, or 21/50 for 0, 25, 3500, or 7000 ppm, respectively); the findings was not observed in females (0/50, 0/50, 0/50, or 1/50 for 0, 25, 3500, or 7000 ppm, respectively) (Table 9).

Table 9. Incidences of non-neoplastic findings in the liver

	0 ppm	25 ppm	3500 ppm	7000 ppm
Males n= 50				
No. of Animals Examined	50	50	50	50
Liver-Amyloid	10	16	17	18
Liver-Hepatocytomegaly	2	6	17*	21*
Females n= 50				
No. of Animals Examined	50	50	50	50
Liver-Amyloid	4	3	9	10

D. Adequacy of Dosing for Assessment of carcinogenicity

Dosing was considered adequate and not excessive based on observations of increased organ weights and histopathological findings. A limit dose of 7000 ppm was used in this study. Increased organ weights were observed in livers and adrenal glands at 3500 and 7000 ppm of both sexes. Increased testis weights were observed in males at 7000 ppm. Gross pathology showed enlarged adrenal glands at 3500 and 7000 ppm of both sexes. Histopathology examination revealed increases of incidence and severity of vacuolation in the adrenal cortex at 3500 and 7000 ppm of both sexes. In the liver, dose-dependent increases of incidence and severity of hepatocytomegaly were observed in males only. In the testis, increases of incidence and severity on hypertrophy and hyperplasia of the interstitial cell were noted in 3500 and 7000 ppm males.

IV. Toxicology

1. Metabolism

Metabolism Study #1: A metabolism and disposition study (MRID 45696511) on [dihydrofuranone-3-^{14}C]BAJ2740 (Spirodiclofen) (>98% radiochemical purity, lot nos. 10682/12, 10682/28, 10682/37; 99.2% chemical purity; lot no. M00156) was conducted using groups of four male Wistar rats given a single 100 or 2 mg/kg bw oral dose or a 14-day repeated

2 mg/kg bw dose of non-labeled material followed by a single dose of labeled material on Day 15. An additional single low-dose group of four female rats was also included. A group of six male rats with bile duct cannulae received a single dose of 1 mg/kg bw for assessment of biliary excretion. Both disposition of the test article and metabolite characterizations were conducted.

Overall recovery of administered radioactivity was an acceptable 89-107%. Plasma radioactivity data showed that absorption was rapid (t_{max} values of 3-4 hrs for low dose and 8 hours for high dose). Excretion data revealed that expired air was an insignificant route of excretion (<0.05% of the administered dose) and that both urine (57-74% for the low dose and 35% for the high dose) and feces (23-31% for the low dose and 61% for the high dose) were major routes of elimination. Consistent with plasma kinetics data, excretion was 90% complete at 48 hours post dosing. Tissue burdens were negligible and revealed no indication of sequestration or tissue specificity. Approximately 11% of the administered dose appeared in the bile as biotransformation products (including a glucuronide conjugate) that collectively contributed up to one third of the radioactivity excreted in the feces. A decrease in urinary excretion of radioactivity with a commensurate increase in fecal excretion of radioactivity, and comparison of AUC values were indicative of decreased absorption at the high dose. Gender-related differences were observed in that female rats tended to exhibit greater absorption and urinary excretion of radioactivity than did males.

Analyses of excreta revealed that the test article underwent extensive metabolism. Parent compound was detected only in small quantities in the feces but not in the urine or bile. Identified components in urine and feces accounted for approximately 59-90% of the administered dose depending on dose regimen. Ten urinary and eleven fecal components were characterized. With few exceptions, metabolite profiles were generally similar qualitatively but varied quantitatively. Greater levels of parent compound were detected in feces from the high dose group which was consistent with the observed decreased absorption. The gender-related difference in absorption/excretion processes in the single low-dose group was reflected quantitatively and to some extent qualitatively in the metabolite profiles. An assessment of the time course of excretion of metabolites via the feces revealed that most of the metabolites (generally >90%) were excreted during the 0-24 hour period following dosing. Qualitatively, the biliary metabolite profile closely paralleled that of the urine with the exception of a glucuronide conjugate in the bile that represented approximately one third of the radioactivity in the bile over a 24-hour period following dosing. The metabolite profile in the bile also lacked several components (glyoxylic acid, dichloromandelic acid, dichlorobenzoic-acid, mandelic acid-cyclohexyl ester) that were detected in the feces; these were likely the result of biotransformation processes within the gut. The metabolism of [dihydrofuranone-3-^{14}C]BAJ2740 (Spirodiclofen) in the rat generally proceeds via formation of an enol which undergoes hydroxylation or is metabolized to a mandelic acid derivative and dichlorobenzoic acid.

This study (MRID 45696511) met both Tier 1 and Tier 2 requirements. Therefore, this metabolism study in the rat is classified **Acceptable/Guideline**, and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

Metabolism Study #2: A metabolism and disposition study (MRID 45696512) examined the effect of 15-week dietary pretreatment (50 or 2500 ppm) of groups of four male and four female rats with BAJ 2740. Following the dietary treatment, the rats were given a single 2 mg/kg bw oral dose of [dihydrofuranone-3-^{14}C]BAJ 2740 (Spirodiclofen) (Batch Nos. 10682/12;10682/28; 10682/3, >98% radiochemical purity) and absorption, excretion, plasma kinetics, and carcass burdens assessed over a 48-hour period. Metabolite characterizations were conducted on urine and feces samples.

Overall recovery of the administered radioactivity was an acceptable 87-103%. Plasma kinetics data and urinary excretion data affirmed that at least ~58-75% of the test material was absorbed. The urine was the major route of excretion and accounted for approximately 56-75% of the administered dose over a 48-hour period. Elimination via the feces accounted for approximately 22-31% of the single oral dose over the same period. Mass balance data and radioactivity recovered in the skin and residual carcass/tissues were indicative of no bioaccumulation even following the 15-week dietary treatment. Metabolite profiles in urine and feces (at 48 hrs) revealed that the test material was extensively metabolized. No parent compound was detected in the urine although nine metabolites were characterized. In the feces, parent compound accounted for 0.38-13.5% of the administered dose while 12 metabolites accounted for 0.17-8.95% (each) of the dose. The dietary pretreatment did not appear to significantly affect the metabolism and disposition of BAJ 2740 (Spirodiclofen) in the rat. Gender-related difference were observed that were independent of the pretreatment regimen. The most notable difference was that the enol metabolite in urine accounted for 4.88-5.41% of the administered radioactivity in male rats and 39.65-52.42% in female rats. In male rats, 3-hydroxy-enol was the major metabolite (~23-34% vs 3.5-4.5% in female). Other minor quantitative and qualitative gender-related differences were observed that involved metabolites representing less than 5% of the administered radioactivity.

This metabolism and disposition study (MRID 45696512) is classified **Acceptable/ NonGuideline**, and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats. The study was a special study utilizing rats receiving a 15-week dietary pretreatment with the test material for the purpose of determining the effects of the pretreatment on the metabolism and disposition of the test material.

Metabolism Study #3: In a rat metabolism study (MRID 45696509), [dihydrofuranone-3-^{14}C] Spirodiclofen (Lot No. 10682-28; >98% radiochemical purity) was administered to 5 male Wistar Hsd/Cpb:WU rats as a single oral (gavage) dose at 3 mg/kg. A single animal was sacrificed at 1, 4, 8, 24, and 48 hours post-dose and subjected to quantitative whole body autoradiography (QWBA) to examine the distribution of radioactivity among tissues.

Based on the recovery of radioactivity in the urine, the dosed radioactivity was readily absorbed and excreted, with 58.2-58.6% of the dose being recovered in the urine by 24 hours post-dose. Data from the QWBA indicated that concentrations of radioactivity were highest in the small intestines (5.691 μg equiv./g) at 1 hour post-dose, followed by liver and renal cortex (1.343-1.831 μg equiv./g). Concentrations in all other tissues (except fat and dental root) were below the concentration in blood. By 4 hours post-dose, the concentrations declined in the small intestines but increased in all other tissues, with excretory tissues (liver, renal cortex, and bladder; 2.285-5.537 μg equiv./g) and fat (2.698 μg equiv./g) having the highest concentrations. Radioactivity in the small intestines continued to decline at 8 hours post-dose, while radioactivity peaked in the bladder and liver (5.460-6.463 μg equiv./g). Radioactivity also continued to remain high (above the concentration in blood) in fat, renal cortex, and dental root. Concentrations in all tissues had declined by 6- to 10-fold by 24 hours post-dose, with concentrations remaining highest in liver, bladder, dental root, and renal cortex (0.262-0.631 μg equiv./g). By the 48-hour interval, concentrations were <LOD (<= 0.003 μg/g) in all tissues except liver and renal cortex (0.009-0.025 μg equiv). There was no evidence of accumulation in specific organs or tissues.

This metabolism study is classified **acceptable/nonguideline** and, in conjunction with the earlier ADME study, satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

2. Mutagenicity

Neither technical spirodiclofen, a formulation, nor major metabolites (BAJ 2740 enol, BAJ 2740 - MA-3OH-cyclohexylester, BAJ 2740-ketohydroxy, BAJ 2740-hexylester or C6-hydroxyester) were mutagenic in *Salmonella typhimurium* when tested up to the limit dose (5000 μg/plate +/- S9) (MRID Nos. 45696702, 45696817, 45696805, 45696818, 45696819, 45696921 or 45696909), respectively. Technical spirodiclofen was also not mutagenic in mammalian cells (MRID 45696614) or clastogenic in cultured mammalian cells (MRID 45696615) and was not clastogenic or aneugenic in the mouse micronucleus assay up to an overtly toxic dose (800 mg/kg) (MRID 45696701). Similarly, the spirodiclofen formulation, BAJ 2740 240 SC was not clastogenic in cultured Chinese hamster lung (V79) cells (MRID 45696821). With the exception of the *in vitro* chromosome aberration assay in V79 cells with the formulation (MRID 45696821), all studies are acceptable and satisfy the FIFRA guideline for mutagenicity testing. MRID 45696821 is unacceptable because purity information was not provided, but can be upgraded when these data are submitted. There are no mutagenic concerns at this time.

Table 10. Mutagenicity Testing with technical spirodiclofen(BAJ 2740)

Study Type	Results
Ames MRID 45696702	Tested in strains TA1535, TA1537, TA1538, TA100, TA98 ± S9 at concentrations up to 5000 ug/plate. **Negative**, classified **acceptable**.
In vitro Mammalian Cell Gene Mutation MRID 45696614	Tested in Chinese Hamster lung fibroblast V79 cells at concentrations up to 300 ug/mL -S9 and +S9. Cytotoxicity was observed at ≥ 15 ug/mL -S9 and 80 ug/mL +S9. **Negative** for mutagenicity and classified **acceptable**.
In vitro Mammalian Chromosome Aberration MRID 45696615	Tested in Chinese hamster lung (V79) cells at concentration 5-80 ug/mL or 0.75-12 ug/mL -S9 or 10-160 ug/mL +S9. **Negative** in clastogenic response. Classified acceptable.
In vivo Micronucleus Assay MRID 45696701	Tested in a mouse micronucleus assay at a dose 800 mg/kg (MTD). Clinical signs and cytotoxicity were seen at 800 mg/kg. **Negative** assay, classified **acceptable**.

Table 11. Mutagenicity Testing with Formulation BAJ 2740 240 SC

Study Type / MRID	Results
Ames MRID 45696817	Tested in strains TA1535, AT1537, TA98, TA102, and TA100 at concentrations up to 5000 ug/plate ±S9. **Negative** for mutagenicity, classified **unacceptable** (no purity information).
In vitro Mammalian Cytogenetics MRID 45696821	Tested in Chinese hamster lung (V79) cells at concentrations up to 0.5 uL/mL, -S9 and up to 0.1 uL/mL +S9. **Negative** for clastogenic ±S9, Classified **unacceptable** (no purity information).

Table 12. Mutagenicity Testing with Metabolite BAJ 2740 Enol

Study Type / MRID	Results
Ames MRID 45696805	Tested in strains TA1535, AT1537, TA98,TA100, and TA102 at concentrations up to 5000 ug/plate ±S9. **Negative** for mutagenicity, classified **acceptable**.

Table 13. Mutagenicity Testing with Metabolite BAJ 2740-MA-3OH-Cyclohexylester

Study Type / MRID	Results
Ames MRID 45696818	Tested in strains TA1535, AT1537, TA98,TA100, and TA102 at concentrations up to 5000 ug/plate ±S9. **Negative** for mutagenicity, classified **acceptable**.

Table 14. Mutagenicity Testing with Metabolite BAJ 2740-Ketohydroxy

Study Type / MRID	Results
Ames MRID 45696819	Tested in strains TA1535, AT1537, TA98,TA100, and TA102 at concentrations up to 5000 ug/plate ±S9. **Negative** for mutagenicity, classified **acceptable**.

Table 15. Mutagenicity Testing with Metabolite BAJ 2740-Hexylester

Study Type / MRID	Results
Ames MRID 45696921	Tested in strains TA1535, AT1537, TA98,TA100, and TA102 at concentrations up to 5000 ug/plate ±S9. **Negative** for mutagenicity, classified **acceptable**.

Table 16. Mutagenicity Testing with Metabolite C6-Hydroxyester

Study Type / MRID	Results
Ames MRID 45696909	Tested in strains TA1535, AT1537, TA98,TA100, and TA102 at concentrations up to 5000 ug/plate ±S9. **Negative** for mutagenicity, classified **acceptable**.

3. Structure-Activity Relationship

Spirodiclofen is a new chemical, no comparable analog is available for analysis of structure-activity relationship. Spirodiclofen belongs to a new chemical class of Tetronic acids. Literature searches conducted on tetronic acid did not show evidence of carcinogenicity or mutagenicity.

4. Subchronic Toxicity

a. Subchronic Toxicity in Rats

In a 14-week feeding study (MRID 45696715), BAJ 2740 (spirodiclofen) (99.1% a.i., batch/lot #NLL 5605-7-8) was administered to Wistar rats (10/sex/dose) via diet at dose levels of 0, 100, 500, 2500, or 12500 ppm (0, 6.6, 32.1, 166.9, or 851.4 mg/kg bw/day for males and 0, 8.1, 47.1, 215.3, or 995.8 mg/kg bw/day for females, respectively) for 14 weeks. Additional groups of 10 males and 10 females were treated with 0, or 12500 ppm of BAJ 2740 for 14 weeks and then observed for reversible effects over 4 weeks. A satellite group of rats (5/sex/dose) was dosed for 4 weeks for immunotoxicological investigations.

There were no treatment-related effects on mortality and clinical signs. During the treatment period, significantly lower body weights were observed at 12500 ppm (-13% in males and -12% in females) compared with controls and slightly lower body weights were observed at 2500 ppm (-4% in males and -7% in females). Body weight gains were significantly lower at 12500 ppm of both sexes compared to controls. During the recovery period, the difference of body weights in the 12500 ppm group became less (-7% in males and -5% in females) compared to controls. Overall food consumption was comparable to the control. Hematology showed significant decreases of leucocyte counts in the 12500 ppm group of both sexes, decreases of thrombocyte counts in males at 12500 ppm and increases of thromboplastin times at 2500 and 12500 ppm of both sexes. Clinical chemistry showed significant increases of alkaline phosphatase (ALP) levels at 2500 and 12500 ppm of both sexes and asparate and alanine aminotransferases (ALT, AST) levels at 12500 ppm of both sexes. Significantly decreased plasma levels of cholesterol and triglyceride were observed at 12500 ppm of both sexes and 2500 ppm males only. Although T3 and T4 levels were comparable to control, significantly increased TSH levels were observed at 12500 ppm of both sexes and in 2500 ppm females. Organ weight measurements showed increases of absolute and relative weights of the adrenal gland at 12500 ppm of both sexes.

Recovery study was conducted in the high dose and control groups only, recovery females had significantly increased erythrocytes, decreased MCV and decreased MCH levels. And relative testis weights were significantly increased in males during the 4 week recovery period.

Microscopic examinations of adrenal glands showed an increase in severity of uniformly small cytoplasmic vacuolation in the cortex of males at doses of 2500 ppm and above and females at 500 ppm and above. At the high dose, males also showed an increase in severity of mixed cytoplasmic vacuolation in the adrenal cortex. In the recovery groups, all animals showed no treatment-related effects. Immunotoxic effect of BAJ 2740 could not be evaluated in this study due to major deficiencies in the submitted data.

The LOAEL for females is 500 ppm (47.1 mg/kg bw/day) based on increased incidence of small cytoplasmic vacuolation in the cortex of adrenal glands. **The NOAEL for females is 100 ppm (8.1 mg/kg bw/day). The LOAEL for males is 2500 ppm (166.9 mg/kg bw/day)** based on increased incidence and severity of small cytoplasmic vacuolation in the cortex of adrenal glands, decreased cholesterol (week 5 and 13), and decreased triglycerides (week 5). **The NOAEL for males is 500 ppm (32.1 mg/kg bw/day).**

This study is classified acceptable/guideline and satisfies the guideline requirements for a subchronic feeding study in rats (OPPTS 870.3100).

b. Subchronic Toxicity Study in Mice

In a subchronic feeding study (MRID 45696711), BAJ 2740 (spirodiclofen) (99.1% a.i., batch/lot #NLL 5605-7-8) was administered to CD-1 mice (10/sex/dose) via diet at dose levels of 0, 100, 1000, or 10000 ppm (0, 15, 164, or 1640 mg/kg bw/day for males and 0, 30, 234, or 2685 mg/kg bw/day for females, respectively) for 13 weeks.

There were no treatment-related effects seen on mortality, clinical signs, body weights, body weight gains, food consumption, and hematological parameters.

At 10000 ppm, clinical chemistry revealed slightly lower cholesterol levels in both sexes; however, only females showed statistically significant decrease. Histopathological examination of livers showed centrilobular hepatocellular hypertrophy and periportal cytoplasmic vacuolation in males and females. Centrilobular hepatocellular hypertrophy were considered adaptive effects and not adverse. Adrenal cortex showed cytoplasmic vacuolation in males and females with increased severity observed in females only. Examination of male sex organs showed an increase in incidence of hypertrophic Leydig cells in testes. This finding was characterized by a foamy cytoplasm of the enlarged Leydig cells and was located in the intertubular interstitium and beneath the capsule. Decreased absolute and relative kidney weights were observed in high dose males.

At 1000 ppm, male mice showed increases in incidences of hypertrophic Leydig cells in the testes. Females showed an increase in incidence of cytoplasmic vacuolation of the adrenal cortex.

At 100 ppm, no significant findings were observed.

Under the conditions of the study, the NOAEL is 100 ppm (15 or 30 mg/kg/day for males or females, respectively) and the LOAEL is 1000 ppm (164 mg/kgbw/day) for male mice based on an increased incidence of hypertrophic Leydig cells in the testes. The LOAEL is 1000 ppm (234 mg/kgbw/day) for female mice based on an increased incidence of cytoplasmic vacuolation of the adrenal cortex.

This study is classified **acceptable/guideline** and satisfies the guideline requirements for a subchronic feeding study in rats (OPPTS 870.3100).

c. Subchronic Toxicity Study in Dogs

In a 90-day oral toxicity study (MRID 45696803), BAJ 2740 (98.6% a.i., batch/lot #06480/0002) was administered to beagle dogs (4/sex/dose) via diet at dose levels of 0, 200, 630, or 2000 ppm (equivalent to 0, 7.7, 26.6, or 84.7 mg/kg bw/day for males and 0, 8.4, 28.0, 81.0 mg/kg bw/day for females, respectively) for 14 weeks.

There were no compound related effects on mortality, clinical signs, food consumption, hematology, urinalysis, and ophthalmoscopic examinations. There was a dose-dependent decreases of body weight gains in both sexes. Significant decrease of body weight gain was observed in males at doses of 630 ppm and above compared with the controls. Increased plasma transaminase activities (ASAT, ALAT) and increased APh and GLDH levels were seen at doses of 630 ppm and above of both sexes. A trend to lower cholesterol was observed in the 630 and 2000 ppm groups of both sexes. The activities of liver enzymes showed an induction of phase I enzymes (cytochrome p-450-dependent monooxygenases (ECOD, EROD, ALD)) in response to BAJ 2740 administration. There was no effects on the phase II enzyme activities (GS-T and GLU-T). The EH was induced at the high dose in females only. Dose-dependent increases of N-DEM, O-DEM, ECOD and ALD levels were seen in both sexes. The changes of liver enzyme activities suggest an induction of hepatic metabolic activity in response to administration of BAJ 2740. These changes are considered adaptive effects and not regard as adverse effects.

Based on dose-dependent relationship, increased relative organ weights were observed in the liver at 630 and 2000 ppm of both sexes, kidney at 2000 ppm of both sexes, pituitary at 630 and 2000 ppm of males, and adrenal gland at 630 and 2000 ppm of both sexes. Decreased relative prostate weight also was observed in males at 630 and 2000 ppm. Histopathological examination revealed treatment-related findings in the liver, kidney, adrenal gland, prostate, testis, and thymus. Hepatocellular cytoplasmic changes, inflammatory infiltrates, and single cell necrosis were seen in females only at the highest dose. Dilation of the proximal tubules of the renal cortex was seen in both sexes at the 2000 ppm. The dilation is considered an adaptive effect since no degenerative changes were seen. In testes, vacuolation and hypertrophy/activation of Leydig cells was observed in males at 630 and 2000 ppm. In addition, degeneration and/or immaturity of the testicular germinal epithelium, oligo- and aspermia of the epididymes, and immature prostates were detected at doses of 630 ppm and above. A dose-dependent increase of cytoplasmic vacuolation of the adrenal cortex was observed in females at all doses and in males at the 630 ppm and above. The adrenal effects were also observed in rat and mouse studies and were considered significant.

The LOAEL for males was 630 ppm (26.6 mg/kg bw/day) based on decreased body weight gains, decreased relative prostate weight, increased relative liver and adrenal weights and histopathology findings in the adrenal glands, testes, and prostates; the NOAEL was 200 ppm (7.7 mg/kg bw/day). The LOAEL for females was 200 ppm (8.4 mg/kg bw/day) based on increased adrenal gland weight (two out of four animals) which coincided with histopathology findings (cytoplasmic vacuoles in the Zona fasciculata of the adrenal glands); the NOAEL for females was not established.

This 90-day oral toxicity study in the dog is considered acceptable (guideline) and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in dogs.

5. Chronic Toxicity

1. <u>Combined Chronic /Carcinogenicity Study in Rats</u>

In a combined chronic toxicity/carcinogenicity study (MRID 45696808), BAJ 2740 (97.6-98.6%, a.i., batch/lot# 06480/0002) was administered to Wistar rats (50/sex/dose) via diet at dose levels of 0, 50, 100, 350, or 2500 ppm (0, 2.0, 4.1, 14.7, or 110.1 mg/kg bw/day for males and 0, 2.9, 5.9, 19.9, or 152.9 mg/kg bw/day for females, respectively) for two years. Additional groups of rats (10/sex/dose) were treated likewise with BAJ 2740 for interim sacrifice after one year.

No significant treatment-related effects were observed in clinical signs and mortality. Significant decreases in body weights were observed in the 2500 ppm group of both sexes compared to controls (↓8-10% for males up to weeks 101; ↓6-7% for females up to weeks 53). Body weight gains were decreased at 2500 ppm of both sexes up to week 3 and recovered thereafter. Blood analyses showed no significant effects in hematological examinations; however, significant increases of alkaline phosphatase (APh) and decreases of cholesterol and triglyceride levels (not statistically significant) were observed in both sexes at 2500 ppm at all test points. Significantly increased thyroxine (T4) levels were observed in 2500 ppm males at weeks 53 and 105. Increased thyroid stimulating hormone (TSH) were observed at 2500 ppm of both sexes but the statistical significance was observed only in females at weeks 79 and 105. Gross and histopathology examinations showed an increased incidence of vacuolated enterocytes in the jejunum (both sexes), increased incidence and severity of vacuolation in Zona fasciculata cells of the adrenal cortex (males only), increased portion of ovarian stroma, and increased incidence of uterus nodules (females) at 2500 ppm.

Under conditions of this study, the LOAEL is 350 ppm for males (14.7 mg/kg bw/day) based on increased incidence of Leydig cell hyperplasia. The LOAEL is 2500 ppm (152.9 mg/kg/day) for females, decreased body weights, decreased body weight gain, increased APh levels, increased TSH, uterus nodules, and increased vacuolated jejunum enterocytes.

The NOAEL is 100 ppm for males (4.1 mg/kg/day) and 350 ppm for females (19.9 mg/kg/day).

2. Carcinogenicity Study in Mice:

In a carcinogenicity study (MRID 45696724), BAJ 2740 (spirodiclofen) (97.6-98.6% a.i., batch/lot # 06480/0002) was administered to CD-1 mice (50/sex/dose) via diet at dose levels of 0, 25, 3500, or 7000 ppm (equivalent to 0, 4.1, 610, or 1216 mg/kg bw/day for males, and 0, 5.1, 722, or 1495 mg/kg bw/day for females, respectively) for 18 months.

There were no compound related effects on mortality, clinical signs, body weight, food consumption, and hematology examinations. Increased organ weights were observed in livers and adrenal glands at 3500 and 7000 ppm of both sexes. Increased testis weights were observed in males at 7000 ppm. Decreased absolute and relative kidney weights were observed in 3500 ppm and 7000 ppm of both sexes. Gross pathology showed enlarged adrenal glands at 3500 and 7000 ppm of both sexes. Histopathology examination revealed increases of incidence and severity of vacuolation in the adrenal cortex at 3500 and 7000 ppm of both sexes. In the liver, dose-dependent increases of incidence and severity of hepatocytomegaly were observed in males only. In the testis, increases of incidence and severity on hypertrophy and hyperplasia of the interstitial cell were noted in 3500 and 7000 ppm males. A dose related increased incidence of amyloid was observed in various organs of both sexes. The lesion in the testis consisted of increased cell size as well as numbers of cells. Additional histopathology findings included discolored testis in mid dose males, epididymis aspermia in high dose males, lymphocytic infiltrate in high dose females and increased incidence of focal opacity in high dose males. **Under conditions of this study, the NOAEL is 25 ppm (4.1 mg/kg bw/day for males and 5.1 mg/kg bw/day for females). The LOAEL is 3500 ppm (610 mg/kg bw/day) for males** based on increased absolute and relative liver and adrenal weights, decreased absolute and relative kidney weight, enlarged adrenal gland, discoloured testis, adrenal gland vacuolization, interstitial cell degeneration of the testes and amyloid. **The LOAEL is 3500 ppm (722 mg/kg bw/day) for females** based on increased absolute and relative adrenal weight, decreased absolute and relative kidney weight, increased incidences of adrenal gland pigmentation, adrenal vacuolization and amyloid.

This carcinogenicity study in the mice is classified **acceptable/guideline** and satisfies guideline requirement for a carcinogenicity study [OPPTS 870.4200b; OECD 451] in mice.

3. <u>Chronic Toxicity Study in Dogs</u>

In a chronic toxicity study (MRID 45696810), BAJ 2740 (97.8% a.i., batch/lot # 06480/0002) was administered to beagle dogs (4/sex/dose) in diet at dose levels of 0, 20, 50, 150, or 500/600 ppm (equivalent to 0, 0.56, 1.38, 4.33, or 16.1 mg/kg bw/day for males and 0, 0.59, 1.52, 4.74, or 17.7 mg/kg bw/day for females, respectively) for 52 weeks. The highest dose was increased from 500 ppm to 600 ppm in study week 4.

There were no compound related effects on mortality, clinical signs, food consumption, body weight gains, urinalysis, hematology, clinical chemistry, and ophthalmoscopic examinations. Liver enzyme activities showed a dose-dependent increases of N-DEM, and O-DEM activities which indicated an induction of hepatic metabolic activity in response to BAJ 2740 administration.

The relative organ weight showed dose-related increases in adrenal glands of both sexes, kidney (females only), and testes, epididymes and prostates in males. Histopathology of the adrenal gland revealed an increased incidence of cortical vacuolation in the zona fasciculata of both sexes at 150 and 500/600 ppm. In the testes, increased incidences of Leydig cell vacuolation, slight Leydig cell hypertrophy, and tubular degeneration were observed in males at 500/600 ppm.

Under the conditions of this study, the NOAEL is 50 ppm (1.38 mg/kg bw/day for males and 1.52 mg/kg bw/day for females) and the LOAEL is 150 ppm (4.33 mg/kg bw/day for males and 4.74 mg/kg bw/day for females) based on increased relative adrenal weights in both sexes, increased relative testis weight in males and histopathology findings in the

adrenal gland of both sexes.

This chronic study in the dog is **acceptable (guideline)** and satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100b, OECD 452] in dogs.

6. Mode of Action

There is no acceptable Mode of Action available.

A package of supplemental data consisting of twelve studies and an integration report submitted to support mode of action (MOA) arguments for Spirodiclofen was submitted and evaluated. The package was considered unacceptable (Memo, K. Hamernik, 7/25/2003, TXR No. 0052003). The reasons are as follows.

Overall the special MOA data package was difficult to follow. The review of the entire package was hampered due to a number of problems, deficiencies and inconsistencies with data reporting and presentation, thus putting the reviewer in the position of trying to figure out what the authors meant and what the implications were for the arguments being put forth. A significant problem encountered was confusing or unintelligible areas in studies due to apparent translation of reports from German to English. Deficiencies included non-referenced statements/assumptions, incomplete study reports and the citation of methods without submission of supporting literature references. The integration document did not compensate for the inadequacies noted in the special studies. See sections below in this memo for more details.

The proposed mode of action is complex and multi-faceted, purporting to explain certain neo- and non-neoplastic effects in endocrine/reproductive tissues of different species (rodent, dog) and sexes and postulating the involvement of a number of biochemical pathways and compensatory/feedback phenomena. It is critical that the MOA position be laid out clearly in a way that the line of reasoning can be readily followed. In reviewing the package, it appeared that possibilities exist for additional MOA components or factors which would require additional consideration for inclusion or exclusion in discussions or arguments.

In addition, a satisfactory MOA argument for the benign and malignant liver tumors observed in the CD-1 mouse was neither proposed in the package nor supported by submitted data or information.

ADDITIONAL COMMENTS:

It should also be noted that the putative MOA for the rodent and dog neo/non-neoplastic findings (excluding mouse liver tumors) involves perturbations in cholesterol regulation. There are classes of human drugs (e.g. statins and others) which interfere with cholesterol and fat disposition so there may be implications for Spirodiclofen with regard to SAR- relationships, human relevance and cumulative risk issues which should be explored.

In addition, the putative MOA involves disruption of steroidogenesis. This is linked to cholesterol as cholesterol is a precursor in steroid hormone synthesis. Drug literature indicates that human hyperlipidemic drugs such as the statins are generally not recommended clinically for females of child bearing age nor for use during pregnancy. Cholesterol and/or steroid hormone disruption could have consequences for the developing organism, therefore, it is recommended that this aspect be considered in the hazard assessment of Spirodiclofen and the evaluation of the main study database.

As a final note, 2,4-Dichloromandelic acid has been identified as a major plant metabolite and was included in experiments in one of the submitted special studies but the information provided was insufficient to assess any potential contribution to the overall hazard potential of Spirodiclofen and other of its metabolites.

V. Committee's Assessment of the Weight-of-Evidence

1. Carcinogenicity

- Male rats had a significant increasing trend at p<0.01, and a significant difference in the pair-wise comparison of the 2500 ppm dose group with the controls at p< 0.05, for testes Leydig cell adenomas. The incidence of Leydig cell adenomas was 2/34 (6%), 1/35 (3%), 0/43 (0%), 4/35 (11%), and 10/43 (23%) for the control, 50, 100, 350, and 2500 ppm dose groups, respectively. The incidence of Leydig cell adenomas of 23% for the high dose group is outside the historical control range of the performing laboratory (2-8%). The incidence of Leydig cell adenomas for the 350 ppm dose group (11% (censored); 4/50 (8%), uncensored), although not statistically significant, was just within the boundary of the historical control range (2-8%) and was considered to be biologically significant. This is supported by an increase (not statistically significant) in focal Leydig cell hyperplasia at 350 ppm **Therefore, The CARC considered the increase in Leydig cell adenomas at 350 and 2500 ppm to be treatment-related.**

- Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 2500 ppm dose group with the controls, for uterine adenocarcinomas and combined adenomas and/or adenocarcinomas, all at p<0.01. The incidence of uterine adenocarcinomas was 4/47 (9%), 5/48 (10%), 3/46 (7%), 2/46 (4%), 14/47 (30%) for the control, 50, 100, 350, and 2500 ppm dose groups respectively. The incidence of combined adenomas and adenocarcinomas was 4/47 (9%), 5/48 (10%), 3/46 (7%), 3/46 (7%), 14/47 (30%) for the control, 50, 100, 350, and 2500 ppm dose groups respectively. The incidence of adenocarcinomas at the high dose (30%) was outside the laboratory historical control range of 2-10% for adenocarcinomas. **The CARC considered the uterine tumors at the high dose to be treatment-related.**

- **The CARC considered dosing at the high dose (2500 ppm) to be adequate and not excessive based on body weight decrease, changes in clinical chemistry, and histopathological findings.** Significant decreases in body weights were observed in the 2500 ppm group of both sexes compared to controls (↓8-10% for males up to weeks 101; ↓6-7% for females up to weeks 53). Body weight gains were decreased at 2500 ppm of both sexes up to week 3 and recovered thereafter. Clinical chemistry findings in both sexes at 2500 ppm included increased alkaline phosphatase, and decreases of cholesterol and triglyceride levels. Significantly increased thyroxine (T4) levels were observed in 2500 ppm males at weeks 53 and 105. Increased thyroid stimulating hormone (TSH) were observed at 2500 ppm of both sexes, but the statistical significance was observed only in females at weeks 79 and 105. Histopathological findings included vacuolated enterocytes in the jejunum (both sexes), vacuolation in Zona fasciculata cells of the adrenal cortex (males only), increased portion of ovarian stroma, and increased incidence of uterus nodules (females) at 2500 ppm.

- Male mice had significant increasing trends in liver adenomas and adenomas and/or carcinomas combined, both at $p<0.01$. There was a significant increasing trend in liver carcinomas at $p<0.05$. There were significant differences in the pair-wise comparisons of the 3500 and 7000 ppm dose groups with the controls for liver adenomas, both at $p< 0.05$. There were significant differences in the 3500 ppm dose group at $p<0.05$ and in the 7000 ppm dose group at $p<0.01$ with the controls for liver adenomas and/or carcinomas combined. The incidence of combined adenomas and carcinomas was 1/46 (2%), 1/50 (2%), 8/47 (17%), and 10/48 (21%) for the control, 25, 3500, and 7000 ppm dose groups, respectively. The incidences of combined liver adenomas and carcinomas of 17% and 21% for the 3500 ppm and 7000 ppm dose groups, respectively, are outside the historical control range of the performing laboratory (4-14%). **The CARC considered the increase in adenomas and combined adenomas and/or carcinomas in the liver at the top two doses to be treatment-related.**

- Female mice had a significant increasing trend, and a significant difference in the pair-wise comparison of the 3500 ppm dose group with the controls, for liver adenomas and/or carcinomas combined, both at $p<0.05$. The incidence of combined adenomas and/or carcinomas was 0/49 (0%), 0/50 (0%), 5/48 (10%), and 3/47 (6%) for the control, 25, 3500, and 7000 ppm dose groups, respectively. The incidences of combined liver adenomas and carcinomas of 10% (statistically significant) and 6% (not statistically significant) for the 3500 ppm and 7000 ppm dose groups, respectively, are outside the historical control range of the performing laboratory (0-2%). **The CARC considered the increase in combined adenomas and/or carcinomas in the liver at the top two doses to be treatment-related.**

- The CARC considered the high dose of 7000 ppm, a limit dose, in the mouse carcinogenicity to be adequate and not excessive. This was based on observations of increased organ weights (liver, adrenal gland, and testes) and histopathological findings (vacuolation of adrenal cortex, hepatocytomegaly, and testicular hypertrophy/hyperplasia of interstitial cells).

2. Mutagenicity

- There is no mutagenicity concern for spirodiclofen.

3. Structure Activity Relationship

- There are no SAR data for spirodiclofen.

4. Mode of Action

- The data, as presented, do not support a Mode of Action for spirodiclofen.

VI. Classification of Carcinogenic Potential

In accordance with the EPA Proposed Guidelines for Carcinogen Risk Assessment (July 1999), the Committee classified Spirodiclofen as **"Likely to be Carcinogenic to Humans"**, based on tumors seen in both sexes of two species (male rat testicular Leydig cell tumors (high dose), female rat uterine tumors (high dose), male and female mouse liver tumors (multiple doses)).

VII. Quantification of Carcinogenic Potential

The CARC recommended that a low dose extrapolation model be applied to the experimental animal tumor data and that quantifications of risk be estimated for male rat testes, female rat uterine, and male and female mouse liver tumors for Spirodiclofen. The most potent unit risk will be used for the purpose of lifetime cancer risk assessment by the Agency.

VIII. Bibliography

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R100710

Chemical:	**Butanoic acid, 2,2-dimethyl-, 3-(2,4-dic**
PC Code:	**124871**
HED File Code	**21200 CARC Reports**
Memo Date:	**06/10/2004**
File ID:	**TX0052552**
Accession Number:	**412-04-0243**